

FIG. 2

The values found are arbitrary in any series of graphs, but they are suitable for comparison either

## ON MOLECULAR ORGANIZATION IN AMEBAN PROTOPLASM

THE question whether a submicroscopic organization exists in protoplasm has been considered with increasing frequency during the past several decades. Among the more recent discussions come to mind those of H. Przibram. R. G. Harrison. L. J. Henderson, J. Needham, E. J. Lund, C. V. Taylor, G. H. Parker, W. T. Bovie and others. The wide-spread and persistent interest in this subject is presumably due to the belief that the proof of the existence of submicroscopic organization would not merely add a new series of facts to science concerning the nature of protoplasm but would, in addition, serve to bring many observations on the form and behavior of organisms into relation which now stand by themselves. And as a consequence, it would also serve as a good tool for further investigation in a number of lines of work.

The data presented herewith seem to me to constitute evidence in support of the hypothesis of molecular organization in the protoplasm of four species of amebas. Other data will also be presented as presumptive evidence tending to show that similar organization may be general among organisms and that certain large groups of hitherto unrelated observations fall into a coherent system under this generalized hypothesis. Since the entire argument is based ultimately on molecular movements and displacements, a statistical treatment is probably the most appropriate method of handling the data.

I. When an ameba is placed on a thin glass rod or within a fine capillary tube of suitable size, the path which the ameba makes on the rod or within the tube by direct inspection or by using them for plotting other graphs showing rates of growth. If it is desired, however, the slope may be translated quickly to absolute rate of growth through reference to a curve, which is obtained by calculating from the growth data the actual rates corresponding to a few representative slopes. Such a curve of reference is shown in Fig. 2. When the slope of  $26.5^{\circ}$ , as found for interval "A" in Fig. 1, is referred to the curve in Fig. 2, it will be seen that this slope corresponds to a growth rate of 1.26 centimeters per day.

The protractor method for determining growth rates is very simple and rapid, and it should be particularly useful when large quantities of growth records have to be studied.

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is a helical spiral of greater or less regularity, depending somewhat upon the individual, the species and other factors. The direction of the path may be to the right or to the left, for varying lengths of time, and may alternate frequently during the course of a few hours. Of the four species investigated, three are predominantly *left*, making each about 1.4 left turns to 1 right; the other species, which readily hypertrophies, is predominantly *right* to a slightly greater degree.

Now if one takes a convenient, small unit of measurement, such as one half turn around the rod or tube, and then measures all the right and left sections of path to the nearest half turn and arranges the sections in a frequency series based on length, a characteristic distribution is obtained for each species. Thus for the 4 species: Rugipes bilzi, Mayorella conipes, Trichamoeba schaefferi, T. sphaerarum, the distribution in the higher categories closely approximates the exponential series represented, respectively, by the formulas:

$$2^{-\left(\frac{y-1}{2}\right)}, 2^{-(y-\frac{1}{2})}, 3^{-(y-\frac{1}{2})}, 4^{-(y-\frac{1}{2})},$$

where y is the length of any section. The data, representing 316, 3250, 322 and 615 sections respectively, of the above named amebas, are shown graphically in the figure. (The points representing the lower categories on the figure, which have drifted off the theoretical curve and are believed to represent the operation of an added factor, will be discussed in a more extended paper to be published soon.) For convenience of graphic representation the observed number of sections are proportionated so that the highest category,  $y^{\circ}$ , equals 1,000 and the log of the

numbers of sections in each category is plotted against the length of the section.

(1) It will be noticed that the first two series noted above are alike numerically but that in the first ameba the steps or nodes in the path where changes of direction are most likely to occur, are twice as far apart as in the second, third and fourth amebas.

(2) The series differ by integral steps.

(3) A particular frequency may occur independently of the particular length of the internode between changes of direction.

In general, these data show that there is a succession of "weak points" or nodes somewhere in the neighborhood of three and one half body-lengths of the ameba apart, for the last 3 amebas, and 7 body-

rods, the number of sections is not the same on both, nor about twice as great on the thin rod, as might perhaps be expected, but there are actually 40 per cent. more sections on the thin rod.

(3) The experimental conditions having been the same in both cases, the results stated in the paragraph above can not have been due to asymmetries in lighting or gravity but must clearly involve some function of the curvature of the rod.

(4) Whatever the length of the node as produced experimentally, the frequency of the breaks (degree of cohesion or field strength) remains the same.

The frequency of changes of direction or field strength of the molecules of the first 2 species of amebas mentioned above is therefore exactly the



lengths for the first ameba. It is at these weak points or nodes that the direction of movement is more likely to change than at any other points. Statistically, the periodicity is remarkably regular.

That the periodicity is not due to the recurring asymmetry of light and geotropic influences as the ameba moves around the rod, is shown in the graph in the upper right corner of the figure. Two rods of different diameter were used in this series of observations, one 59 microns and the other 120 microns in diameter.

(1) The frequency formula, each based on one half turn of the rod as a unit of measurement, is exactly the same for both rods, in the higher categories. This would be expected mathematically.

(2) For equal amounts of space covered on the 2

same, but the nodes are twice as far apart in the first as in the second ameba. But in the second, third and fourth amebas the nodes are the same distance apart but the field strength of the molecules differs systematically.

II. Another set of experiments bearing on molecular organization is concerned with the effect of light. Two of the above named amebas, *conipes* and *sphae-rarum*, were subjected to the same technique as described above, excepting that the light was alternated in some experiments every 30 minutes, in others every 45 minutes, in others still, every 60 minutes, with light of lower or higher intensity. The intensity ratios, varying from 2 to 8, were secured by use of a light wedge, sector wheel or distance difference. For the purpose of this discussion amebas may be divided into 2 classes: a larger class in which the total number of left turns is greater than the number of right turns; and a smaller class in which the right turns similarly predominate. It has been found experimentally that amebas predominating in left turns react to changes of light intensity that is exactly opposite and approximately equal to the reactions of predominantly right amebas.

(1) In both types of amebas belonging to the species *conipes*, the average change of the left-right ratio of spiral turns in 5 different pairs of intensity alternations, comprising 2,000 turns, was found to be 18.6 per cent. more right turns in the *higher* intensity for the *left* amebas; and 18.4 per cent. more right turns in the *lower* intensity for the *right* amebas.

(2) In sphaerarum, using 3 pairs of intensity alternations and comprising 1,009 turns, 22.4 per cent. more right turns were produced in the *higher* intensity by predominantly *left* amebas, while 24.1 per cent. more right turns were produced in the *lower* intensity by the *right* amebas.

(3) In the light of physical science these results, obtained by varying the light intensity, are interpreted as due to the action of light-activated atoms in the molecules, which causes these molecules to change their spiral twist from left to right or vice versa, depending upon the conditions of the experiment. But if these light effects are considered in connection with the periodicities and internodes, all of which are related integrally, then only one conclusion seems possible with respect to the nature of the underlying mechanism: it must be particulate and therefore molecular. This means, further, that high correlation between a structural or functional characteristic of an organism and a specific left-right ratio of spiral turns is a sign of a corresponding stereorelation between the atoms in the spiral molecule. In other words, high correlation between a specific leftright ratio and the presence of any characteristic of an organism means that the characteristic is a steric property of the spiral molecule. (The converse is of course not true, since there must be many steric displacements possible which do not throw the molecule as a whole from a left spiral twist into a right, or vice versa.)

Seeing that the spiral molecule is labile enough to be easily changed from one direction of twist to another by merely changing the light intensity one would naturally expect other physical or chemical agencies to produce similar changes. In illustration of such expectation two correlations, numbered III and IV are given immediately below. Following these are two other correlations, numbered V and VI, which point to a probable and very interesting statistical relationship between two very wide-spread characteristics of organisms and their distinctively steric characteristics.

III. Two of the species of amebas referred to above, *bilsi* and *schaefferi*, frequently hypertrophy markedly under some cultural conditions, when they become strongly right-turning and reproduction is rare or absent.

IV. A normally left-turning *sphaerarum* becomes strongly right-turning within 30 minutes after eating a large and easily digestible food object, and does not become predominantly left-turning again until after several hours have elapsed.

V. The direction of spiral twist of body in animals is negatively correlated, statistically, with the direction of spiral movement; in plants the correlation is apparently positive.

All motile organisms move spirally when guiding senses are not functioning, so far as known. By experiment and observation this has been found to hold true from bacteria to blindfolded aviators. Many organisms have spirally twisted bodies. Many others have some prominent unpaired organ spirally twisted. Many others, again, show no conspicuous spirality. The lack of spirality in the body of an organism is, however, not correlated with its property of moving spirally; for this class of organisms moves as definitely spirally and to the same degree as does the other class. The mechanism of spiral movement is, therefore, independent in its presence and in its function of the gross or visible spiral structure of the body. But there is a statistical correlation between the *direction* of the visible spiral structure and the *direction* of spiral movement.

(1) Of the 168 species of ciliate protozoa (Bullington), 146 belong to right-spiral structure groups and 22 to left-spiral structure groups. Of the 146 species belonging to right-spiral structure groups, 104 swim to the left spirally and 42 swim to the right. Of the 22 in the left-spiral structure group, 20 swim to the right and 2 swim to the left.

(2) Of 40 species of flagellates studied quantitatively, belonging mostly to the Euglenoidinea, almost if not quite all *show* left-spiral structure and all were found to swim to the right.

As stated above, many organisms swim spirally in which no spiral structure has yet been found; but if one uses the data derived from the study of the 208 species of ciliates and flagellates, which show definite negative correlation between direction of spiral structure and spiral movement, then the 102 species of rotifers studied, of which 92 swim to the right and 10 to the left, may be said to constitute a left-spiral structure group. Likewise also the 22 species of tunicates and salpas, which have been found to swim to the right, may be said to constitute a left-spiral structure group. In addition to these data may be mentioned the snails, of which 15,230 are right spiral in structure and about 730 left (Leunis), but no observations have been found recorded as to direction of spiral movement of larvae or adult of any snails. Three hundred species of dinoflagellates are structurally left spiral but the movements of only about a dozen have been studied carefully and of these, half are right-turning, one left, and the rest alternate from right to left, but the predominance of the last group is not recorded. Volvox and some of its congeners are known to swim to the left, as do also the larvae of about 40 species of worms, bivalves, echinoderms and coelenterates all that have been studied.

Summarizing, it is seen that groups of organisms with left-spiral structure (assumed in rotifers) swim to the right predominantly in the ratio of about 10 right to 1 left, or greater; while right-spiral structure groups swim left predominantly in the ratio of about 2.3 left to 1 right.

In plants, however, the situation is different. The cyanophyceae and spirochaets are twisted to the left and the motile species swim to the left. The spermatozooids of the characeae, mosses and ferns, so far as recorded, swim in the same direction as the body is twisted. Among the spermatophytes, 48 out of 65 climbing plants turn to the right (Darwin, Cook). From the few observations recorded, motile plants are evidently unlike animals in that the plants move in the same direction as their bodies are twisted, but they agree with animals in that the lowest groups are almost all left in structure and the highest predominantly right in structure.

VI. The incidence of sex among the different groups of animals is also statistically correlated to a greater or less degree with right-spiral structure, and apparently also in plants. Taking first the left-spiral groups among animals, we find, with only a very few exceptions, asexual reproduction among the flagellates; very great reduction in the size of the male among the Vorticellidae and the rotifers, and hermaphroditism among the tunicates. The large groups closely allied to these, respectively, are right spiral in structure and sexual equality is the rule. Of the living gastropods about 43 per cent. of the species belong to the (hermaphroditic) Euthyneura and 57 per cent. to the (unisexual) Streptoneura (Leunis). Approximately 10.4 per cent. of the Euthyneura are twisted in a left spiral, while less than .2 of 1 per cent. of the Streptoneura are left in structure. Observations on about 40 species of worms, echinoderms, bivalves, crustacea and vertebrates, which constitute the remainder of definite data on this subject, indicate that these groups are predominantly right spiral in structure, since they move spirally to the left; the data are, however, too few in this particular case to do much more than excite a lively interest in collecting more data.

Among the plants the same condition apparently holds. The observations are few and difficult to make, requiring first the development of a new technique for the ready detection of spirality. The lower plants; spirochaets, cyanophyceae, spirillum, are left in structure and sexless. The spermatophytes, as judged by the climbing plants, noted above, are preponderately right in structure and sexual. But the spirogyras are nearly all left in structure and reproduce sexually, although parthenogenesis is also present.

Now taking together all the observations on the relation of sex to spiral structure, involving a number of groups of animals and plants, totaling about 17,500 species, of which the most of course are gastropods, there is seen to exist a comparatively high degree of correlation between the presence of left spiral structure and (a) absence of sex, or (b) marked reduction of sexual equality, or (c) hermaphroditism, on the one hand; and right spiral structure and equality of sex on the other. In the light of the preceding experiments and observations, this points to the very interesting possibility that sex is also fundamentally a molecular property, usually associated with the right stereoisomeric molecule.

We have here then 6 sets of observations which are definitely interrelated statistically. At the bottom of these relationships lie gross spiral structure and spiral movement. Of the 6 sets of data mentioned here, the first 2 sets, on periodicity and light effects are experimental and of course the most definite in pointing to the molecule as the mechanism underlying the observed reactions. The other sets of general observations, so far as they go, support this conclusion, and they are just such correlations as one would expect to find if the general principles of stereochemistry hold also in stereo-biology.

Now an interpretation broad enough to include all these data requires that the protoplasm of amebas, and apparently of other organisms as well, be assumed to consist primarily of molecules, specific chemically for the species, which are organized into definite patterns. The molecules must consist apparently of two fundamental types: right stereoisomers and left stereoisomers, which are not merely spatially different, but like the other stereoisomers of chemistry, they must also differ in some degree in other respects. (Many other stereoisomeric conditions less deep-seated than these could of course exist.) These isomers, in the case of organisms other than amebas, may give rise, most conspicuously, to a spiral structure of the body, which in many cases is more or less masked; second, the right isomer readily falls into the two sexual phases, while the left does so comparatively rarely.

The organizing process of molecules at the forward end of the moving ameba is, in consequence, assumed to be accompanied by the formation of fields which derive their characteristics from the nature of the molecules; for there can be no (spontaneous) molecular organization without an accompanying field (comprising sub-fields). This field is the "organized aspect" of the organism. It follows from this that not only some but most or all of the characteristics of the organism are due to, or rather correlated with positional relationships of the molecules, and ultimately with the stereo-relations of the atoms of the molecules. It is, in fact, theoretically unique to suppose that a mass of matter, of whatever size, in a system (such as a molecule or an organism) can give rise to a particular characteristic of that system; for the characteristics of such systems as we know about are commonly held to be due to number and positional relationships of the constituent particles.

The experimental data on the amebas, on which the hypothesis of molecular organization is specifically based, will soon be published in full.

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## CERCOSPORELLA HERPOTRICHOIDES FRON, THE CAUSE OF THE COLUM-BIA BASIN FOOTROT OF WINTER WHEAT

DURING the past eleven years an important footrot of winter wheat and barley has been under investigation in eastern Washington and Oregon. The symptoms already have been described in part.<sup>1</sup> An undetermined fungus, producing sterile, moderately slowgrowing, compact, smoke-gray, mycelial colonies on Difco potato-dextrose agar, has been consistently isolated from young lesions that occur in the basal nodes of the culm. In special studies with this fungus conducted by the writer during the fall and winter of 1929 and again in the fall of 1930, at Corvallis, Oregon, the fungus sporulated profusely when grown on cornmeal and incubated outdoors. The fungus proved to be Cercosporella herpotrichoides Fron. Conidia were produced in slimy, pink masses, or pseudopionnotes, at the edges of colonies four weeks old. Conidia were developed also on loosely formed coremium-like structures, on sporodochia or microsclerotia, and to some extent on independent hyphae. The spores germinated at one or both ends and, in

<sup>1</sup>H. H. McKinney, "Footrot Diseases of Wheat in America," U. S. Dept. Agr. Bull. 1347: 28-30, 1925. less than a week, produced the characteristic, smokegray colonies on Difco potato-dextrose agar.

Early in the spring of 1930, spores of *Cercosporella herpotrichoides* were found on lesions at the bases of culms of naturally infected wheat plants in the field near The Dalles, Oregon, and also on artificially inoculated wheat plants in the greenhouse at Corvallis, Oregon.

Artificial inoculations on wheat were made, in 1929 and 1930, in the greenhouse and in the field at Corvallis, Oregon, and in the field at three different points in and near The Dalles, Oregon. The fungus was carried through five mono-mycelial transfers before starting the pathogenicity tests. As inoculum for pathogenicity tests, the fungus was grown in quantity in flasks on a sterilized mixture of oats and barley kernels. In making the inoculations the fresh

TABLE 1 RESULTS OF INOCULATING WHEAT PLANTS WITH Cercosporella herpotrichoides at Corvallis and The Dalles, Oregon, 1929-1930

Location	Distance from nearest known naturally in- fected area, miles	When inoculated	Results obtained
Corvallis, Ore.: In green- house	120	At time of seed- ing, Oct., 1929, to Feb., 1930 Oct., 1930, to Jan., 1931	Typical footrot symptoms and also stunting and frequent death of seed- lings
On college farm	120	Jan. 3, 1930, 1 month after seeding	Typical footrot symptoms, in- cluding falling of culms
The Dalles, Ore.: In the coun- try, eleva- tion 2,800 feet In the coun- try, eleva- tion 2,200 for 2,200	2	Oct. 10, 1929, 6 weeks after seeding Oct. 10, 1929, 6 weeks after seeding	Typical footrot symptoms ex- cept no fall- ing Typical symp- toms, including falling
In a gar- den in The Dal- les, eleva- tion 150 feet	10	March 15, 1930, 5 months after seeding	Footrot lesions near maturity